DECREASED SEROPREVALENCE FOR *TOXOPLASMA GONDII* IN SEVENTH DAY ADVENTISTS IN MARYLAND

MARY-CLAIRE ROGHMANN, CHARLES T. FAULKNER, ALAN LEFKOWITZ, SHARON PATTON, JEFF ZIMMERMAN, AND J. GLENN MORRIS, JR.

*Veterans Affairs Medical Center and Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; Department of Comparative Medicine, University of Tennessee, Knoxville, Tennessee; Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa*

**Abstract.** Despite its widespread prevalence, uncertainties remain about the relative contribution of various routes of transmission to the overall rate of infection with *Toxoplasma gondii*, particularly in developed countries. To explore the hypothesis that meat consumption is an important risk factor for infection, a cross-sectional seroprevalence study was performed on healthy adults in one region in the state of Maryland. The population included Seventh Day Adventists who as a group follow a diet containing no meat, and control community volunteers who were not Seventh Day Adventists. Thirty-one percent of the population had serologic evidence of *T. gondii* infection. People with *T. gondii* infection were older (49 versus 42 years old; *P < 0.01, by t-test) and less likely to be Seventh Day Adventists (24% versus 50%; *P < 0.01, by chi-square test) than people without *T. gondii* infection. When adjustments were made for age and gender through multiple logistic regression, Seventh Day Adventists had a significantly decreased risk of *T. gondii* infection (odds ratio = 0.21, 95% confidence interval = 0.09–0.46, *P = 0.0001*) compared with the controls. While the basis for this effect remains to be determined, one possible protective factor is the general adherence of Seventh Day Adventists to a diet that does not contain meat.

The transmission of *Toxoplasma gondii* to humans occurs through the accidental ingestion of sporulated oocysts in the environment or tissue cysts from inadequately cooked meat. There are multiple studies linking exposure to cats or cat feces to *T. gondii* infection.1–5 The risk of transmission from undercooked meat is more controversial,6–10 although biologically plausible given the high prevalence of tissue cysts in uncooked meat intended for human consumption.11 The U.S. Department of Agriculture has estimated that 50% of the *T. gondii* infections in the United States are acquired by eating meat or poultry,12 an estimate that leads to a cost for foodborne toxoplasmosis of $2.7 billion per year of a total of $5.4 billion/year in medical costs and productivity losses associated with the disease.13 If these estimates are accurate, toxoplasmosis is responsible for higher costs than any other foodborne pathogen, including *Salmonella* and *Escherichia coli* (O157:H7).12

Given the increasing recognition of the importance of foodborne diseases in the United States and the need to prioritize resources available for control of these illnesses, there is a need to explore the accuracy of the above estimates, and, in particular, to carefully assess the relationship between meat consumption and *T. gondii* infection. As a first step in this process, we looked for evidence of *T. gondii* infection in a serum collection from a group of Seventh Day Adventists and community controls who were not Seventh Day Adventists. Seventh Day Adventists are advised, for religious purposes, to adhere to a lacto-ovo-vegetarian diet containing no meat, seafood, or poultry. If meat is an important vehicle for acquisition of *T. gondii* in a developed country, it is plausible that Seventh Day Adventists church members would have a lower prevalence of infection than persons who were not Seventh Day Adventists.

**METHODS**

**Study population and sample collection.** We used serum samples that had been collected as part of an evaluation of the possible association between eating shellfish and *Vibrio* and Norwalk virus infections.14 The study was restricted to healthy community volunteers more than 18 years old, and was approved by the Institutional Review Board of the University of Maryland School of Medicine. Seventh Day Adventists volunteers were from six congregations on the eastern shore of Maryland. They were recruited into the study and data were obtained immediately after church services. The control population was from the same area and consisted of commercial fisherman, seafood processing plant workers, and visitors to a seafood festival who were not Seventh Day Adventists. Subjects were given a short self-administered questionnaire on basic demographic characteristics. After appropriate informed consent was obtained, approximately 10 ml of blood was drawn from each subject, and serum was stored in aliquots at –20°C until tested. Since the initial study focused on possible shellfish-associated pathogens, specific data relevant to toxoplasmosis were not collected, i.e., consumption of red meat, poultry, and pork; ownership or contact with domestic cats; or history of outdoor activities. We also lacked data on whether participants were raised in the Seventh Day Adventist tradition since birth, or had converted as adults.

**Serology.** Serum samples were analyzed for antibodies to *T. gondii* by the modified agglutination test using formalin-fixed tachyzoites as antigen.15 Titers ≥ 32 were considered positive.

**Statistical analysis.** Categorical variables were compared with a Pearson’s chi-square test and continuous variables with a Student’s *t*-test. Multiple logistic regression models were used to estimate the association of Seventh Day Adventists status with *T. gondii* infection adjusting for age, race, and gender. First-degree interactions were tested. Age was modeled as both a categorical and continuous variable. Statistical analysis was performed using the SAS statistical package (SAS Institute, Carey, NC).

**RESULTS**

**Characteristics of the study population.** The study population consisted of 262 people. The mean age was 44 years.
Toxoplasma gondii infection by Seventh Day Adventist (SDA) status in study population stratified by age, gender, and race

<table>
<thead>
<tr>
<th>Stratification by age</th>
<th>SDA</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 years</td>
<td>45</td>
<td>79</td>
<td>124</td>
</tr>
<tr>
<td>40–60 years</td>
<td>27</td>
<td>50</td>
<td>77</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>33</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>105</td>
<td>247</td>
<td>352</td>
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</table>

<table>
<thead>
<tr>
<th>Stratification by age</th>
<th>SDA</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 years</td>
<td>19</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>40–60 years</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>10</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>33</td>
<td>31</td>
<td>64</td>
</tr>
</tbody>
</table>

**Table 2**

Multiple logistic regression on the risk of Toxoplasma gondii infection in the Caucasian population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seventh Day Adventists</td>
<td>0.21</td>
<td>0.09–0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age in 10-year intervals</td>
<td>1.49</td>
<td>1.19–1.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.51</td>
<td>0.71–3.22</td>
<td>0.28</td>
</tr>
</tbody>
</table>

* CI = confidence interval.

**DISCUSSION**

Several studies have investigated the transmission of T. gondii infection. Cats are the only definitive host, and, between Seventh Day Adventists and controls in each age group, gender, and within Caucasians. The percentage of both Seventh Day Adventists and controls who were positive for T. gondii increased significantly with age (P < 0.01, by chi-square test for trend). There was no difference between men and women in T. gondii infection among either Seventh Day Adventists or controls. There were too few African-Americans in the Seventh Day Adventist group to justify an estimate of this status on T. gondii infection in African-Americans. Among the controls, there was no difference in T. gondii seropositivity between African-Americans and Caucasians.

Because less than 1% of the Seventh Day Adventists were African-American, an accurate odds ratio (OR) for T. gondii infection in Seventh Day Adventists compared with the controls could not be calculated for African-Americans. Therefore, multiple logistic regression (Table 2) was performed only on Caucasians (n = 189). Both Seventh Day Adventist status and age were independent risk factors for positive T. gondii serology in the Caucasian population. When age and gender were controlled, Seventh Day Adventist had a protective OR of 0.21 (P < 0.01) for T. gondii infection compared with the controls in the Caucasian population. When Seventh Day Adventist status and gender were controlled, the odds of T. gondii infection increased 49% (P < 0.01) for each 10 years of age.
Therefore, the only animal that sheds oocysts in their feces. Humans are infected by ingesting the infective oocysts from contaminated soil or soiled items or by ingesting tissue cysts in raw or undercooked meat. Outbreaks of *T. gondii* infection have been linked to inadequately cooked lamb and hamburger. More recently, *T. gondii* infection was positively associated with eating raw caribou in pregnant Inuit women. However, the relative importance of the food-borne route of transmission in the epidemiology of the disease remains controversial, with at least one study showing that vegetarians had the same seroprevalence as controls who ate meat.

Our results show that Seventh Day Adventists are at a significantly decreased risk for *T. gondii* infection compared with a population of control Caucasians from the same limited geographic area after controlling for age, a known risk factor for *T. gondii* infection. One plausible explanation is that Seventh Day Adventists have a decreased exposure to the organism related to their diet, which excludes pork, beef, shellfish, and poultry. A previous study in Seventh Day Adventists showed a similar seroprevalence of 21.6% for *T. gondii*, but there was no control group for their population. A study of Orthodox Jews showed a prevalence of 48% but did not have an age-matched control group. The strength of our study is the ability to control for geographic area and age, both of which have been associated with *T. gondii* infection in humans.

At the same time, there are clear limitations with the current study. Since these sera were originally collected to detect antibodies related to shellfish pathogens, we lack specific data on consumption of meat (including consumption of raw or undercooked meat) by the participants. According to a local physician and practicing Seventh Day Adventist at one of the congregations, approximately half of the congregation adhere to a population of control Caucasians from the same limited geographic area. A previous study in Seventh Day Adventists showed a prevalence of 48% but did not have an age-matched control group.

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In this context, our data highlight the need for further epidemiologic studies of Seventh Day Adventists and other special dietary groups within the population to better characterize the risk of food-borne *T. gondii* infection in the United States. If these studies substantiate the hypothesis that avoidance of meat results in a significant reduction in risk of infection, attention will need to be given to documentation and possible control of *T. gondii* infection in meat animals or meat products.

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**REFERENCES**